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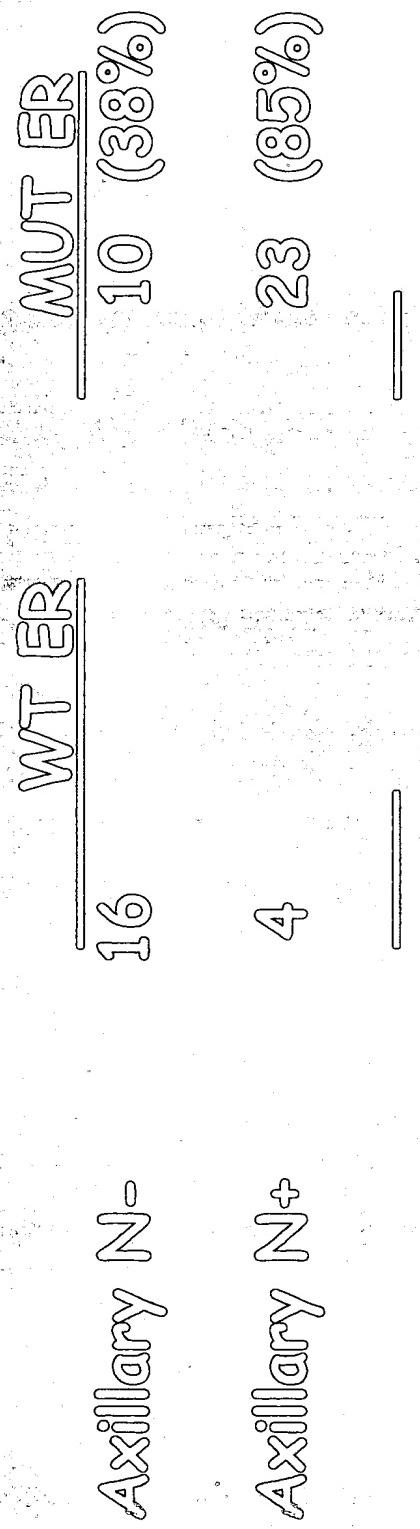
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Frequency of the K303P Mutation Stratified by Nodal Status

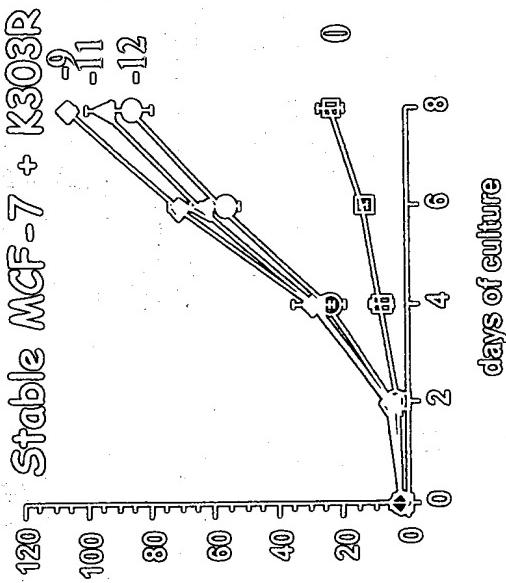
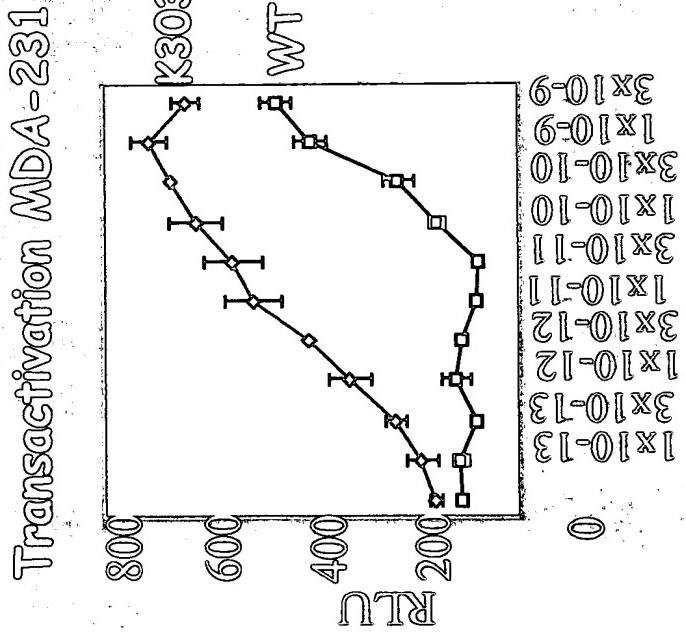
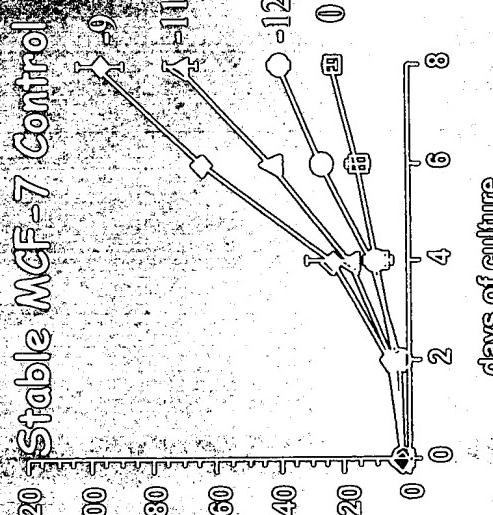


p=0.00062, Fisher's Exact Test

The Mutation is Associated with
Advanced Breast Cancer

Hypothesis:
Proliferative Advantage is due to Hypersensitivity to Estrogen

Growth Assay



The K303R ER α -Mutation is Frequently Present in Postmenopausal, ER α -positive Patients

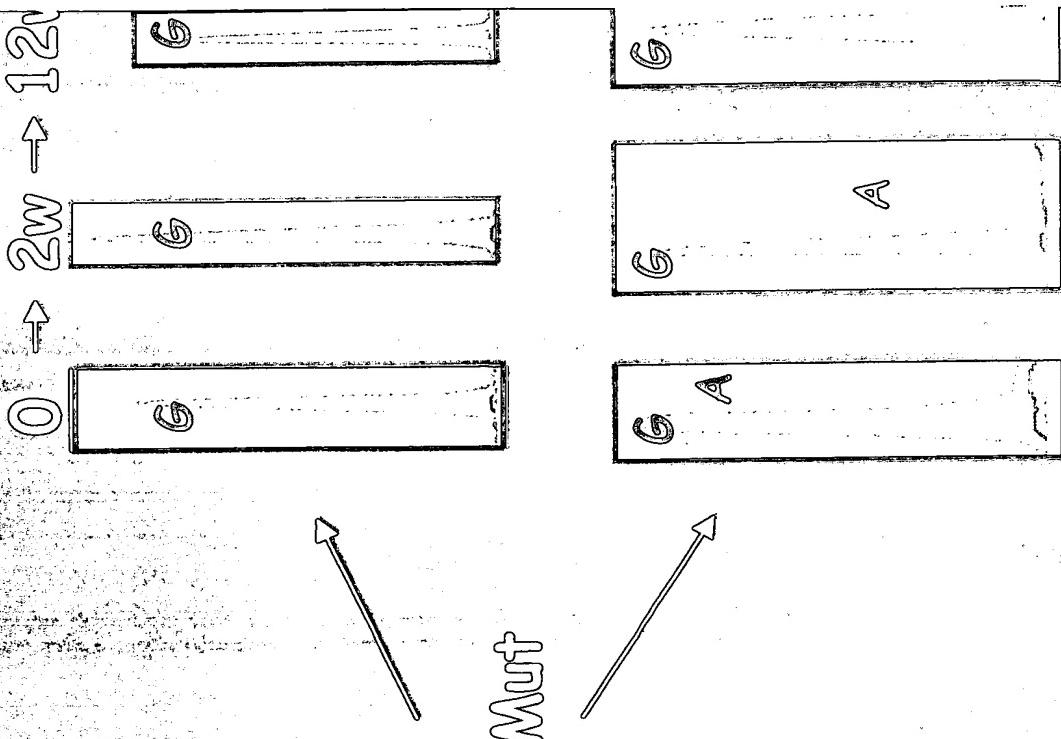
Neo-adjuvant study of 30 primary invasive breast cancers from UK**:

Node-negative

Postmenopausal

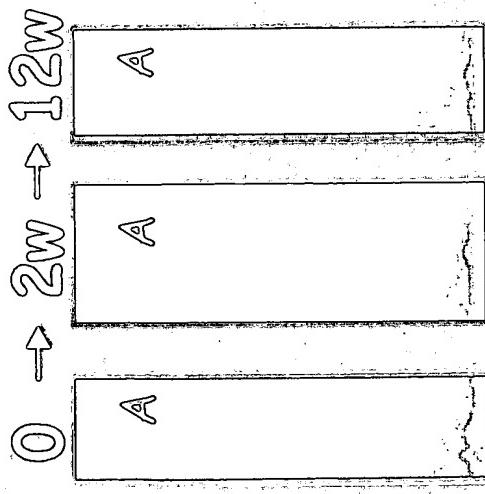
Size > 2 cm

ER α -positive



90% Mut

10% VWT

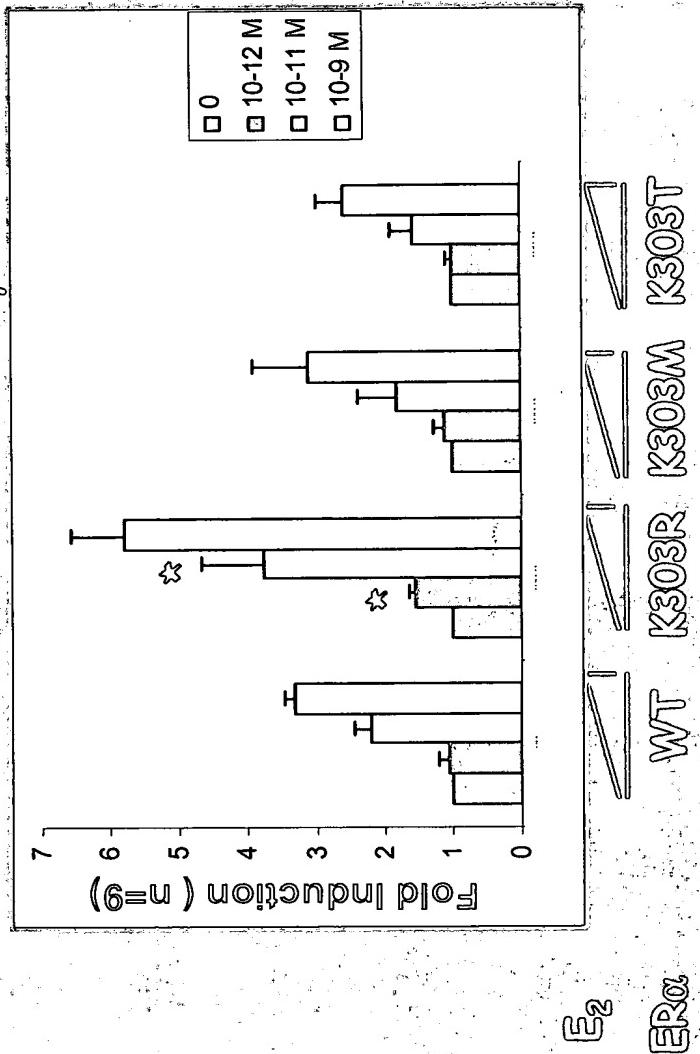


** M. Dowsett

Why Find Only A to G Mutation in Tumors?

A to G = K303R
A to T = K303M
A to C = K303T

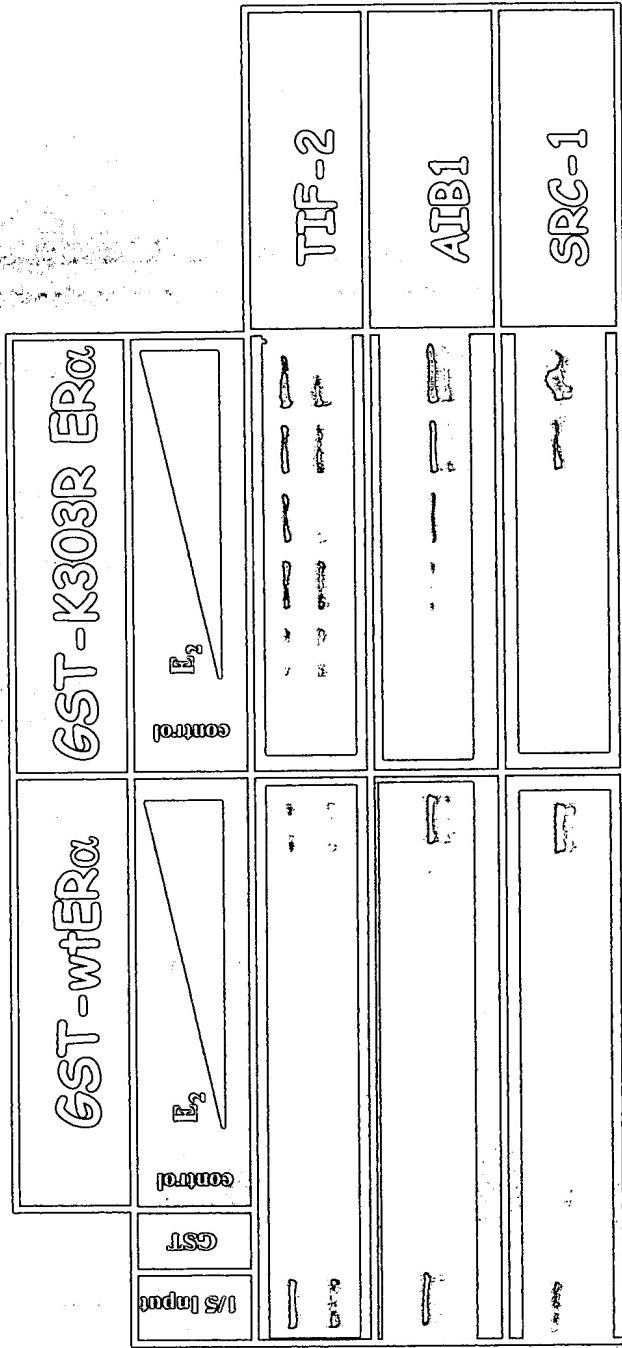
Transactivation Assay



Only K303R is Hypersensitive

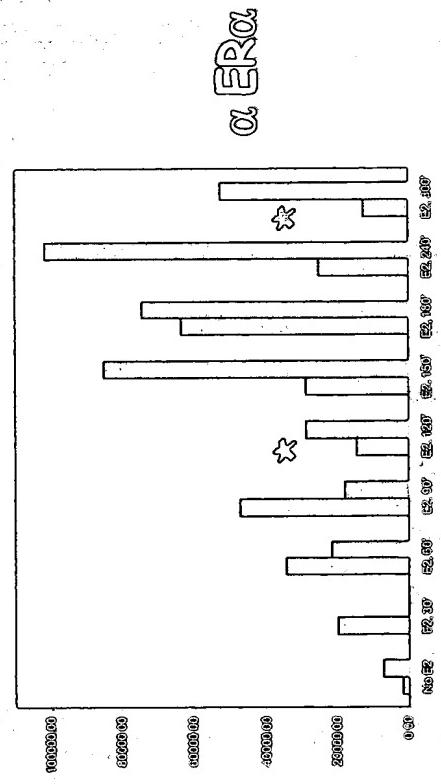
The ERα Mutation Exhibits Altered Binding to
TTF-2 and AIB1 ER Co-activators

GST Pull-Down Assay

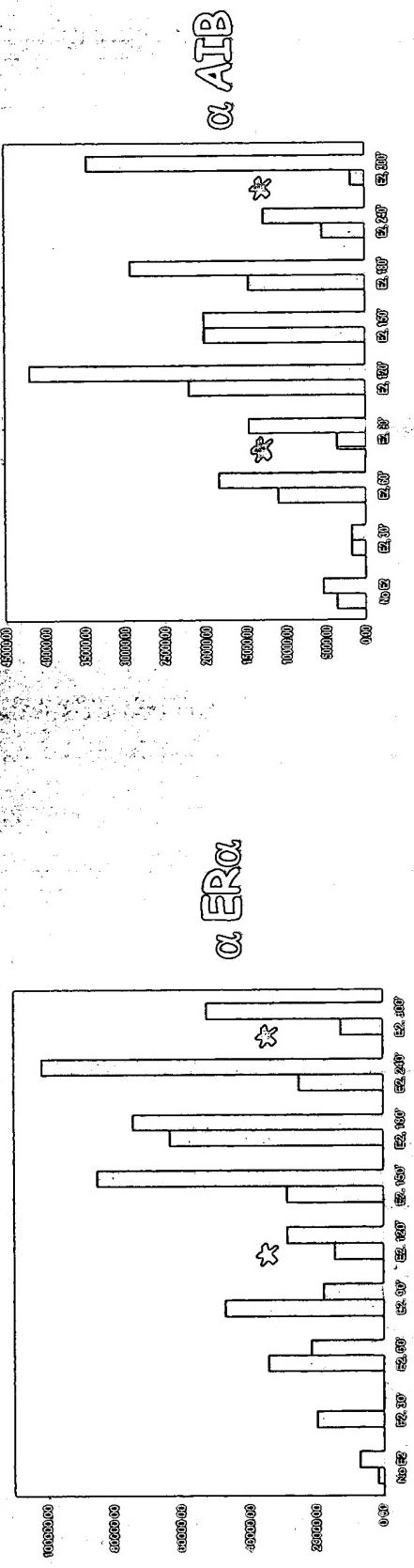


Altered Dynamics of K303R ER α on the pS2 promoter

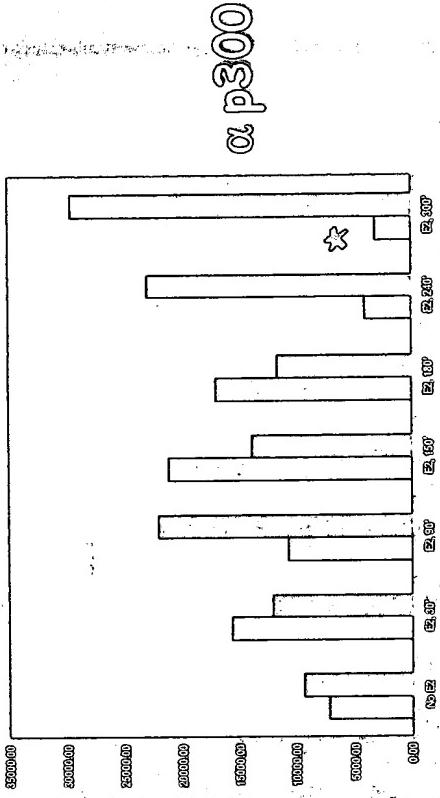
WT



K303R

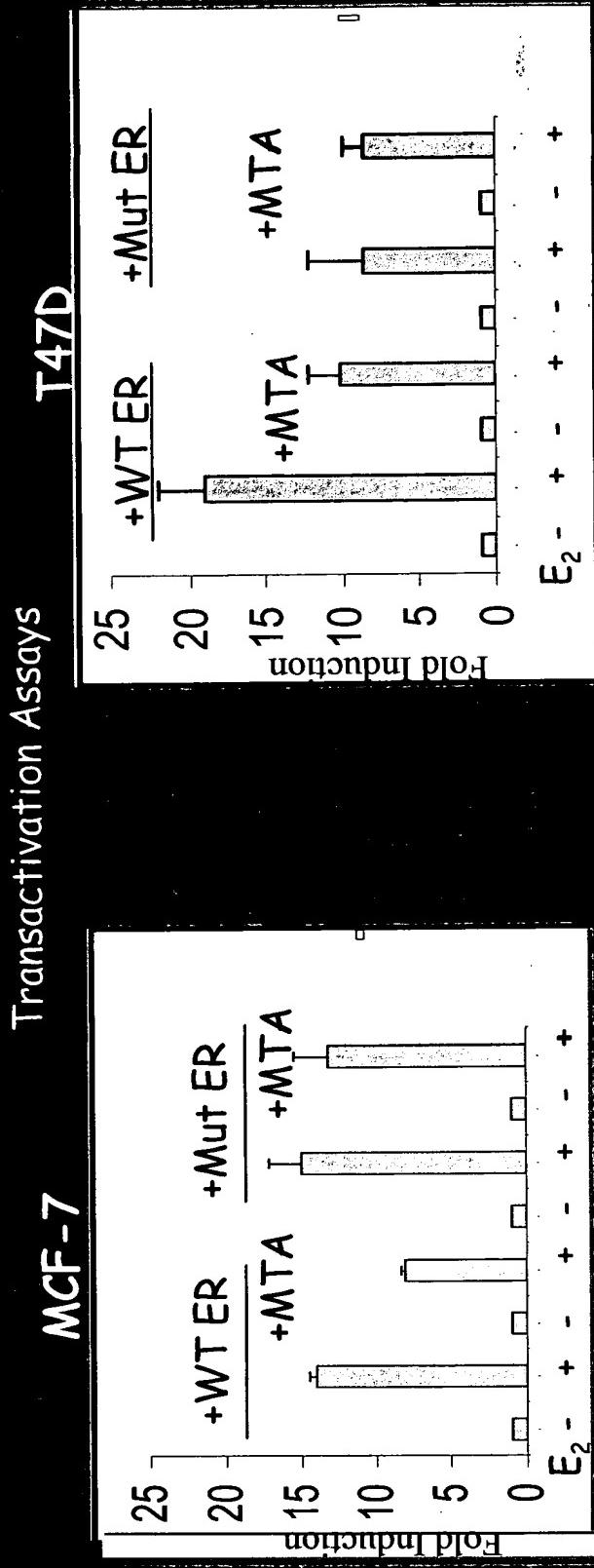


Mutant: increase in estrogen-induced occupancy on the promoter

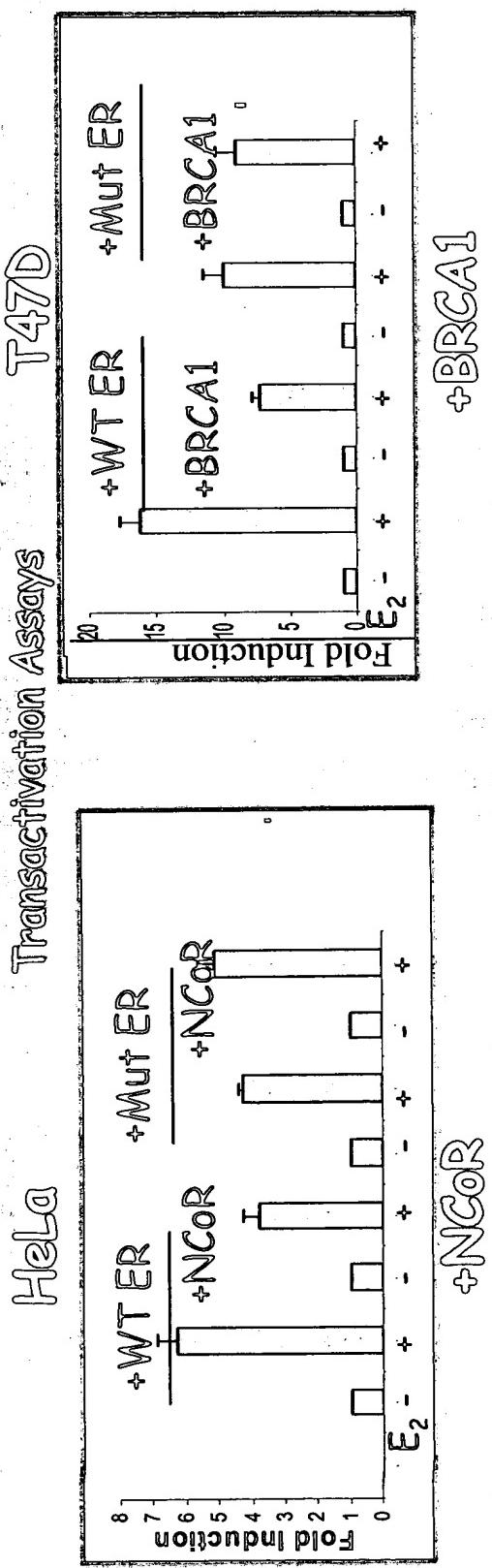


Mutant: p300 appears to be retained in the complex

The K303R ER α Mutant is Resistant to MTA2 Co-Repressor Inhibition Of ER α Activity



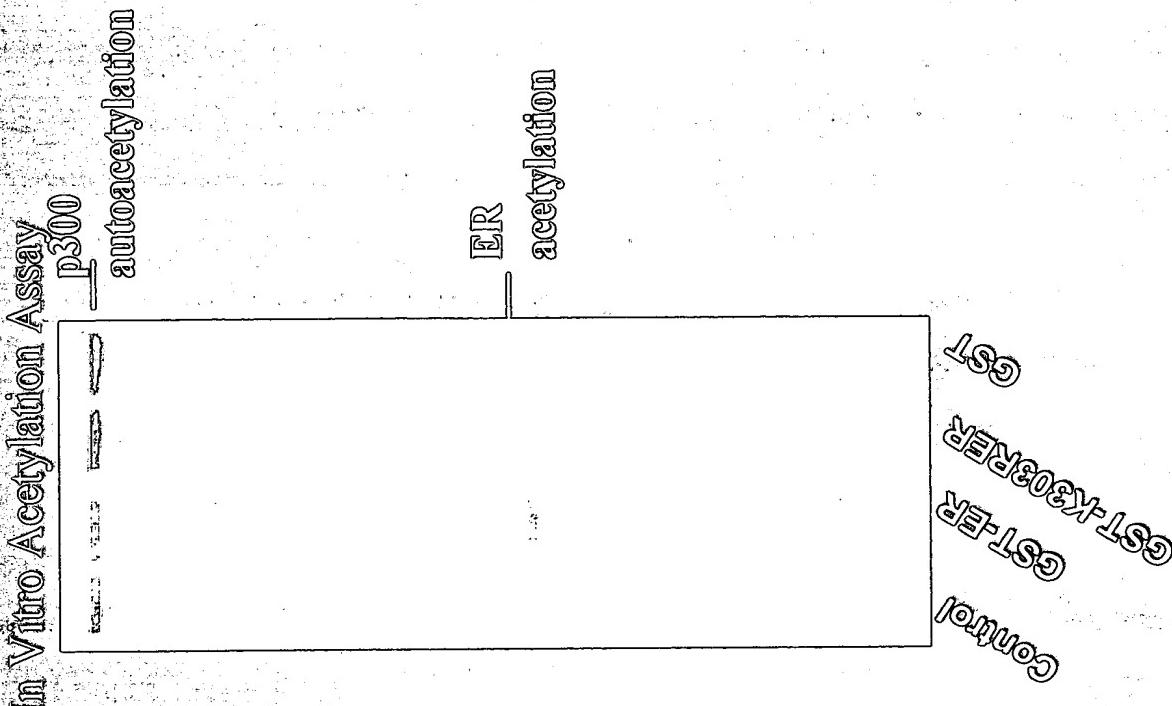
K303R ER Mutant is Resistant to NCoR and BRCA1 Co-Repressor Inhibition of ER α Activity



The K303 ERA Site is Acetylated by p300

Acetylated by HATs
(p300 & P/CAF)

3, GATA-1 motif
R X K K



Acetylated by p300,
not P/CAF

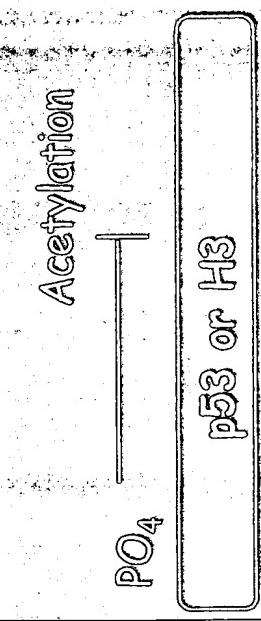
WT ER motif
R S K K

Acetylation by p300
is blocked

Mutant 303 R — +

Wang et al., JBC. 2001

Are Receptor Acetylation and Phosphorylation Coupled Events?

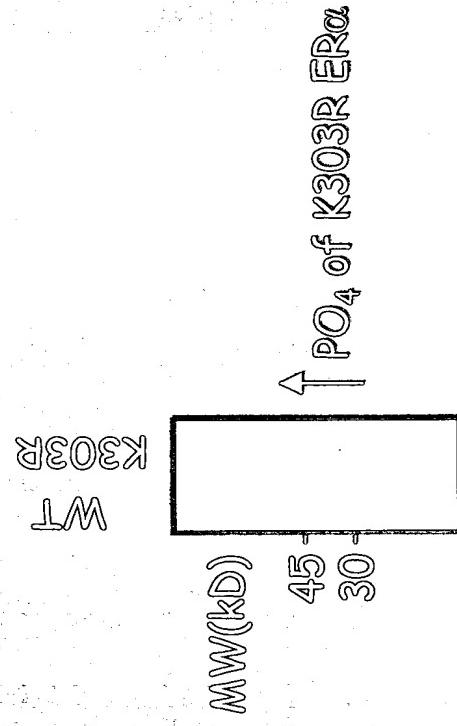


Total *In Vitro* Kinase Assay:

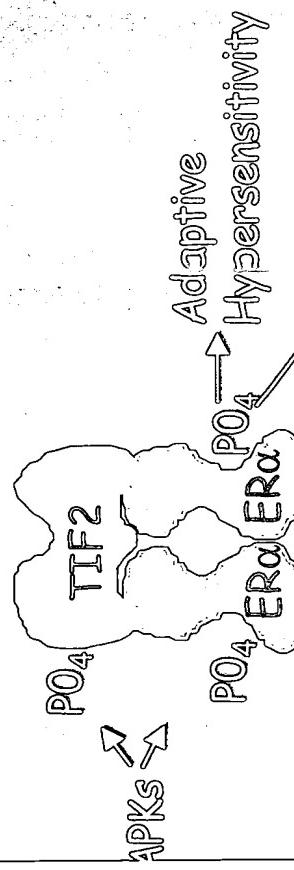
GST-ERs Hinge #253-310

+
MCF-7 Lysate

+
 $\gamma\text{P}32$



Acetylation??



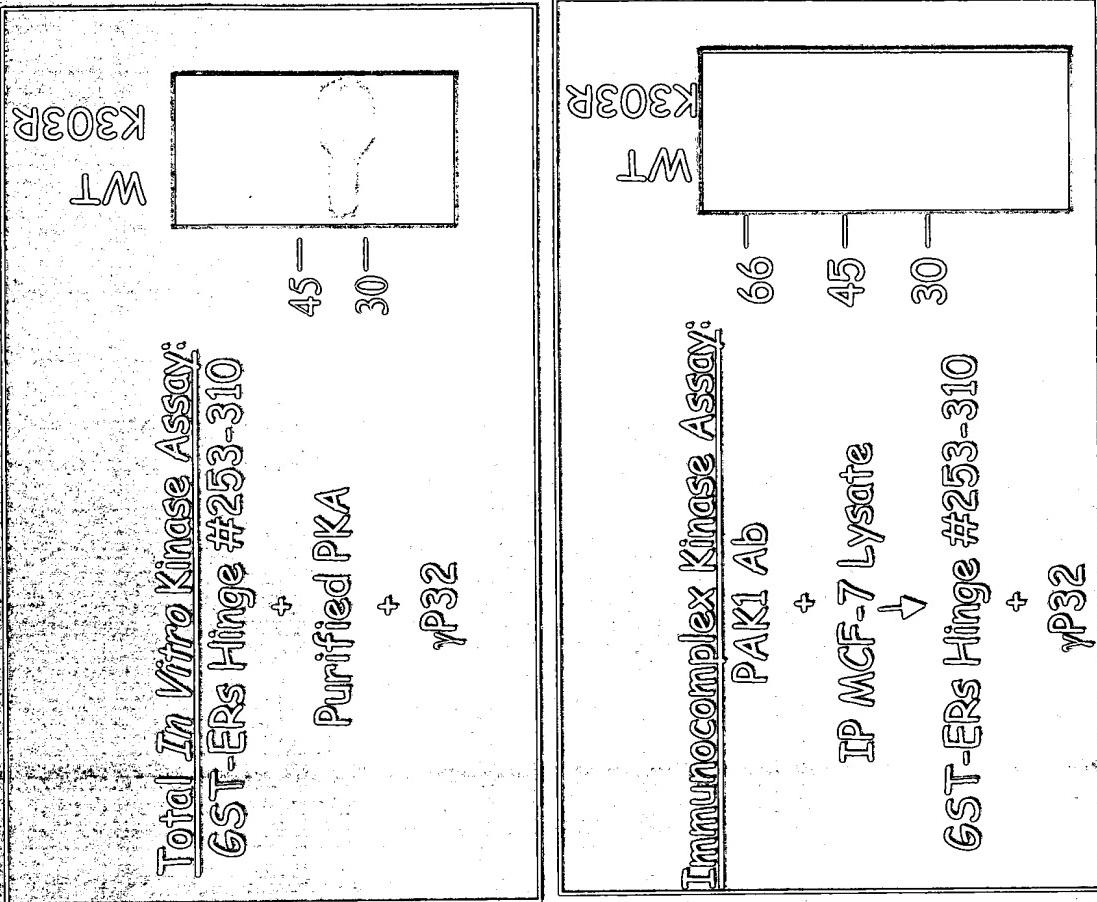
ER Coomassie
blue staining

K303R ERα Mutation Generates an Efficient PKA & PAK1 Phosphorylation Site

Substrate Specificity

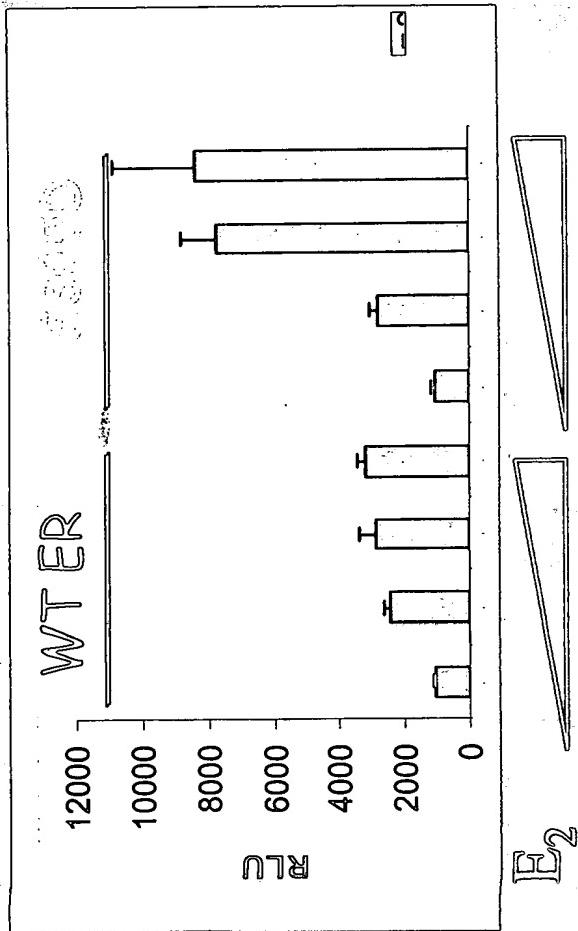
Kinase	RxxS (100%)	KxxS (1%)
PAK1	PAK1	PAK1
WT ER:	KKNS ₃₀₅	
K303R:	KRNS ₃₀₅	

Tuazon, et al. Biochem 1997



ER α S305 Phosphorylation Reduces Receptor Acetylation

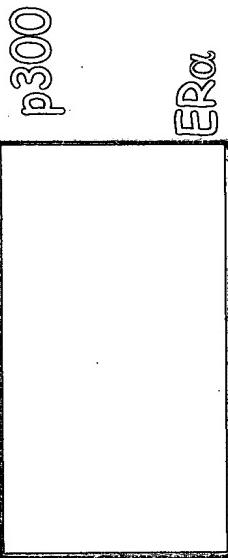
Transactivation Assay



Acetylation Assay: GST-ER α Chime #253-310)

↓
Acetylate with p300

GST-S305D
GST-K303R/S305D
GST-S305A
GST-K303R/S305A
GST-WT
GST-K303R
GST-p300



* Acetylation

Coomassie
blue stain

Other examples where phosphorylation blocks p300 acetylation:
S305 increases estrogen sensitivity
Positive co-activator PC4, Forkhead TF FOXO 3a



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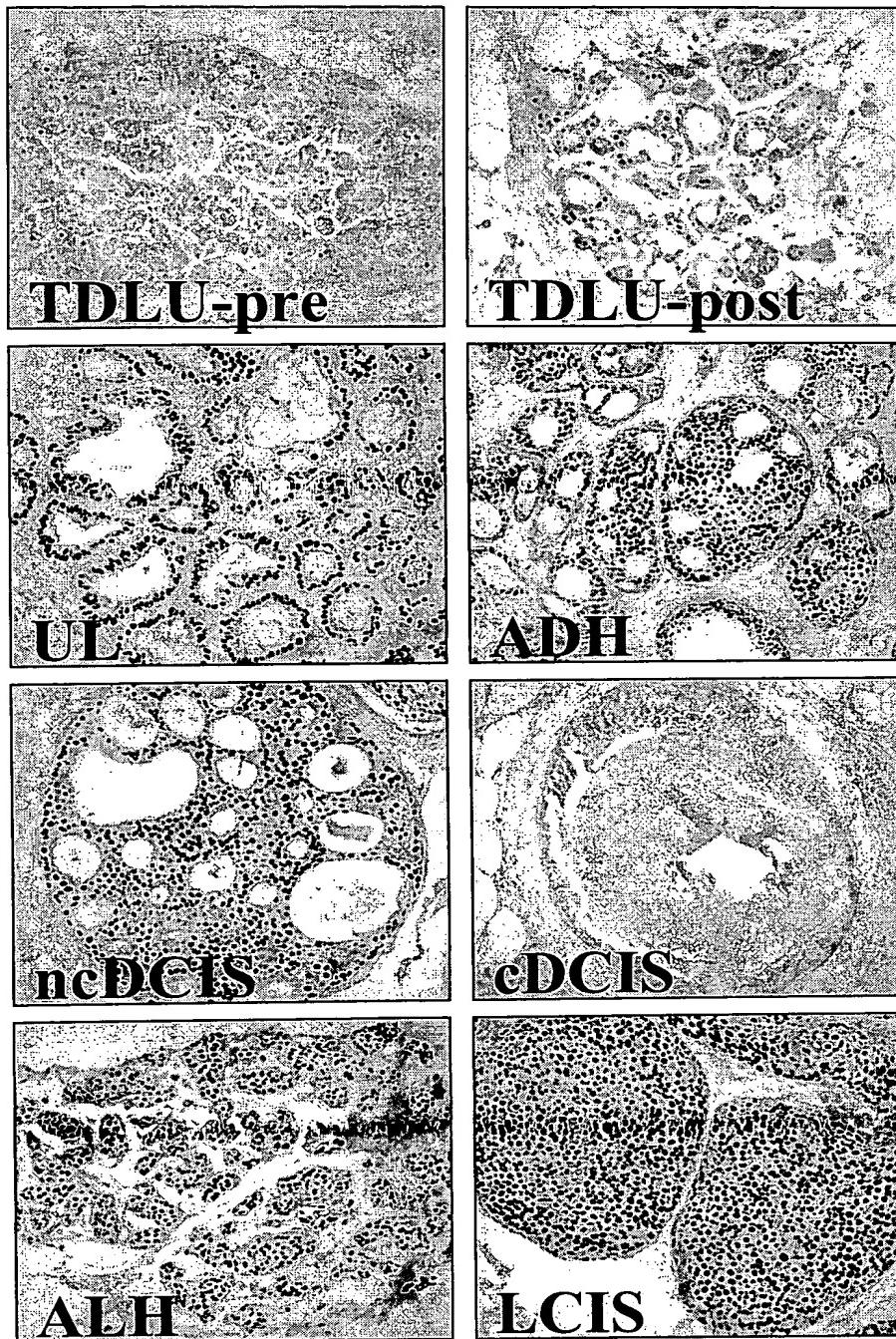


FIG. 1



REPLACEMENT DRAWING

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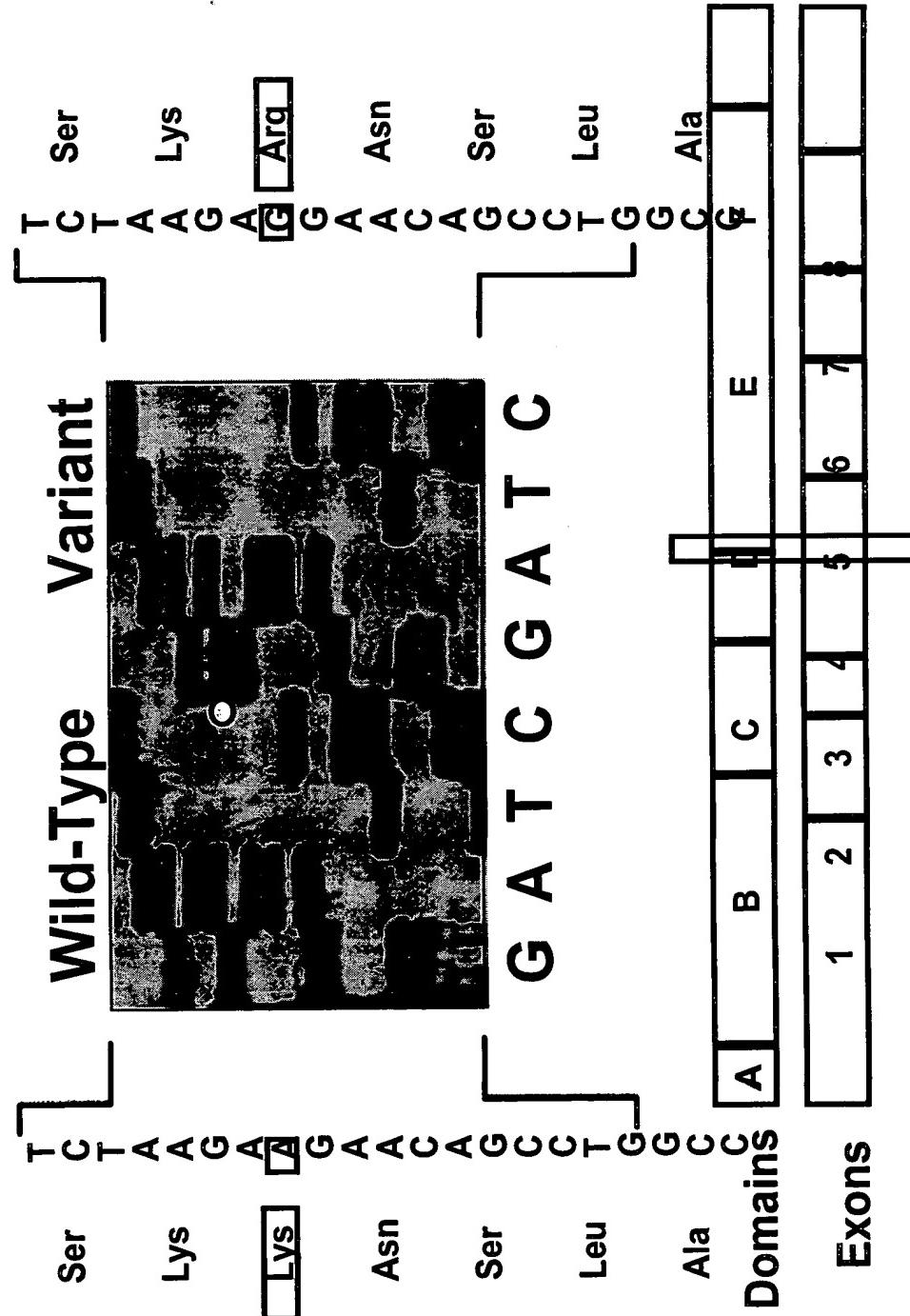


FIG. 2



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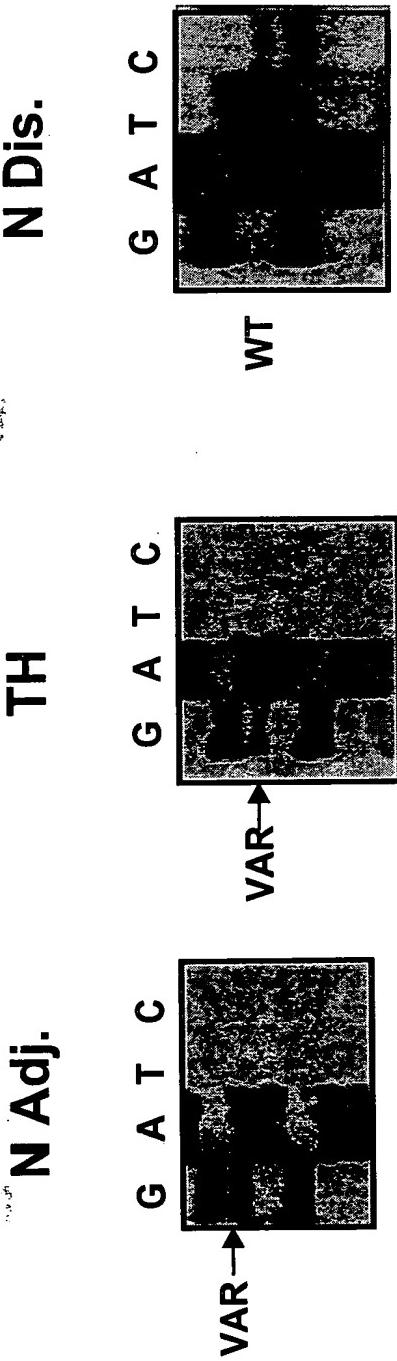
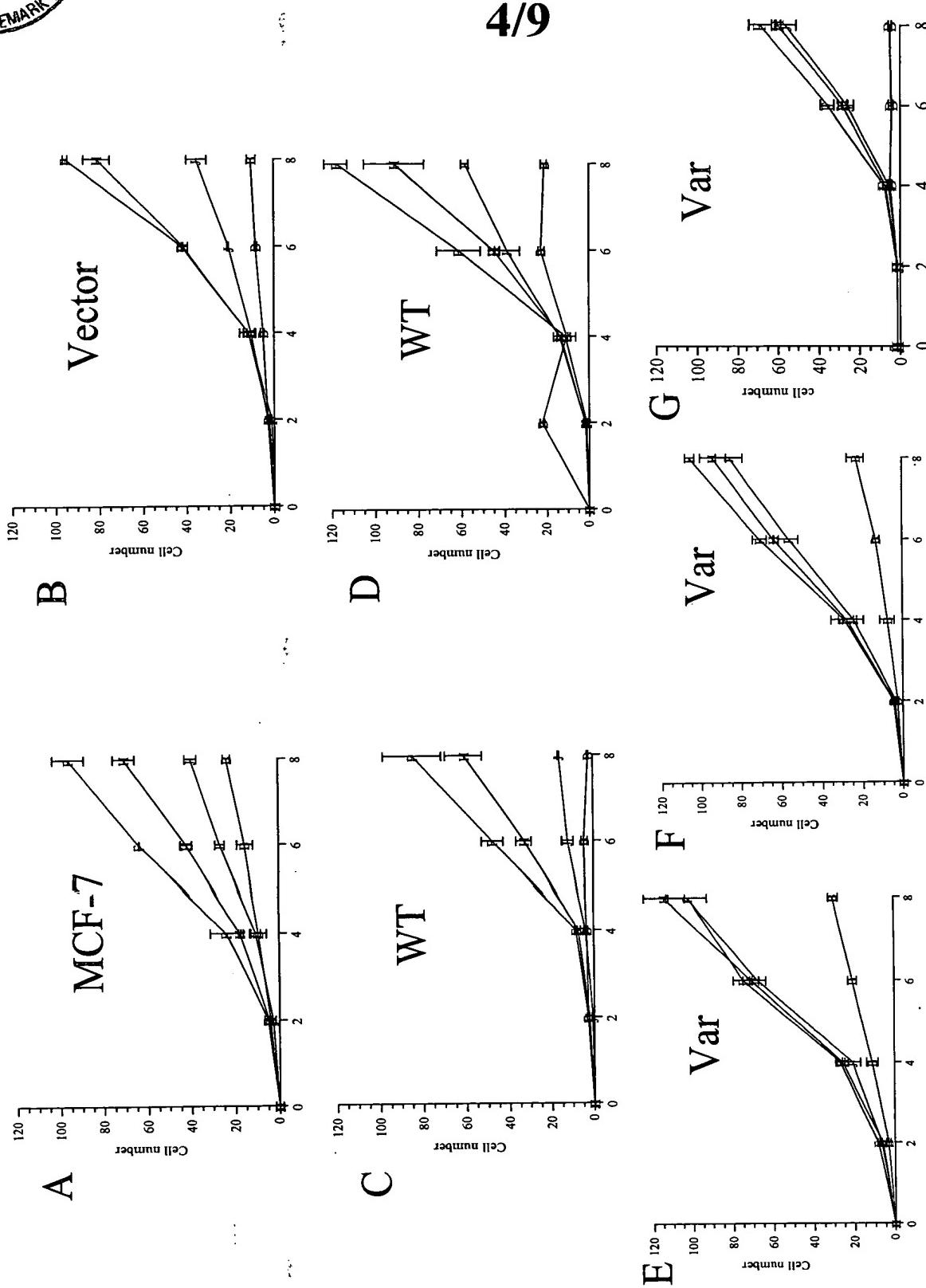


FIG. 3



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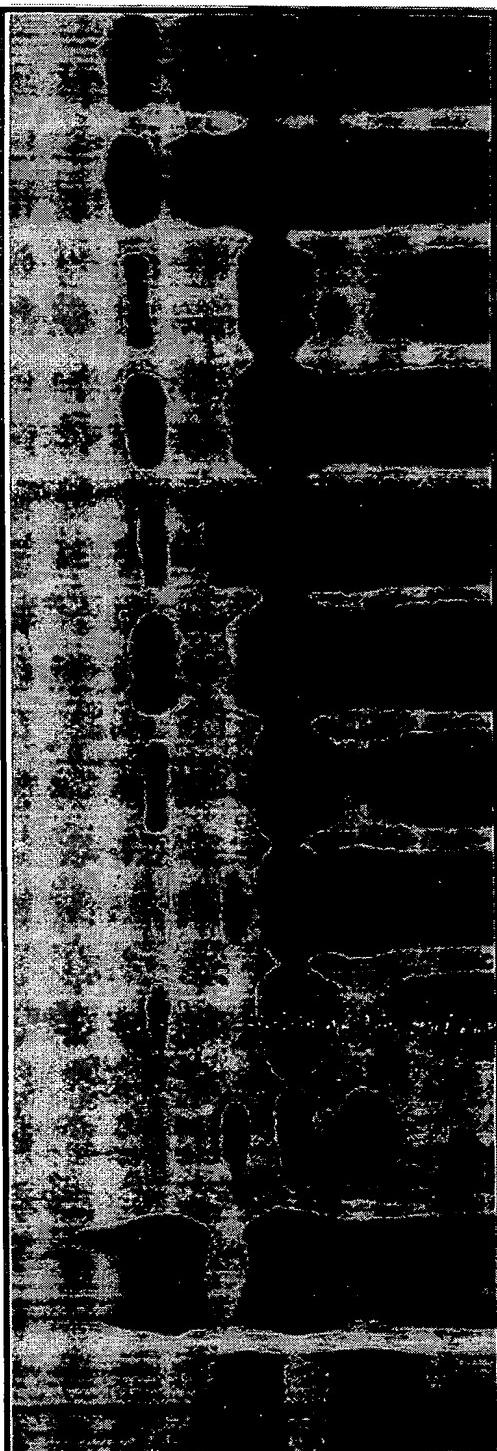
GST-wtER α	GST-K303RER α		SRC-1	SRC-2	SRC-3
1/S Input	GST	control	E_2	E_2	E_2

FIG. 5



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Mut WT 1 2 3 4 5 6 7 8 9 10



WT -
Mut -
WT -
Mut -

FIG. 6



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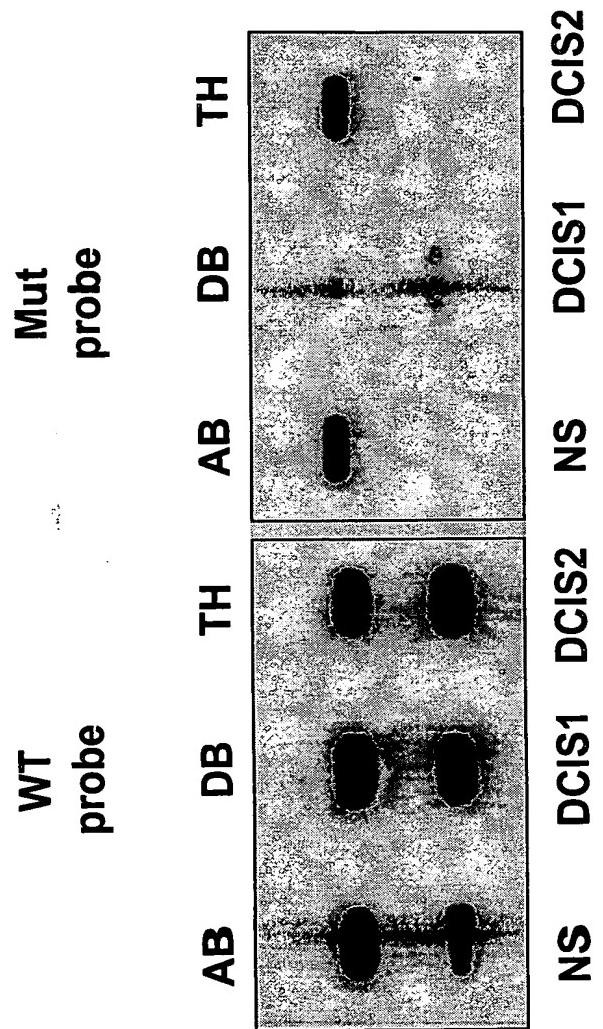
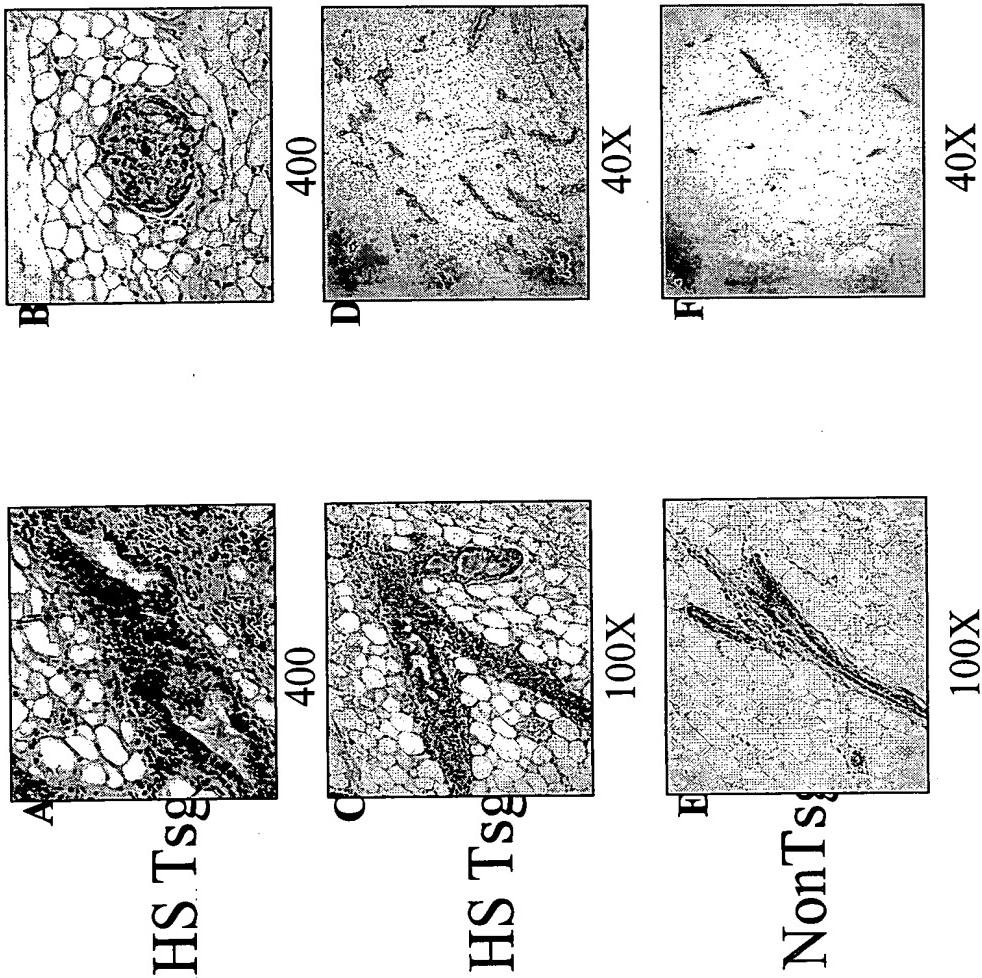


FIG. 7



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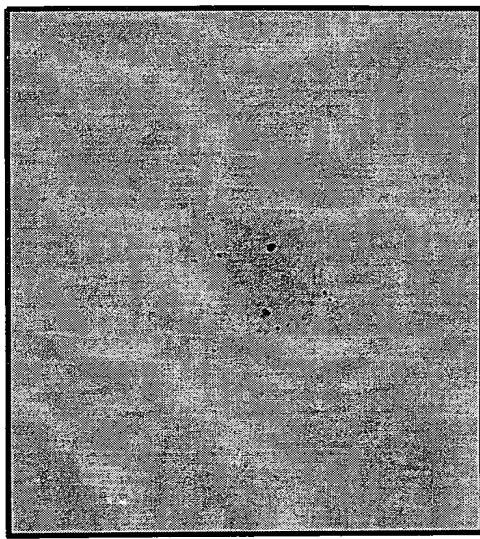
18 month old virgin mice - H&E

FIG. 8

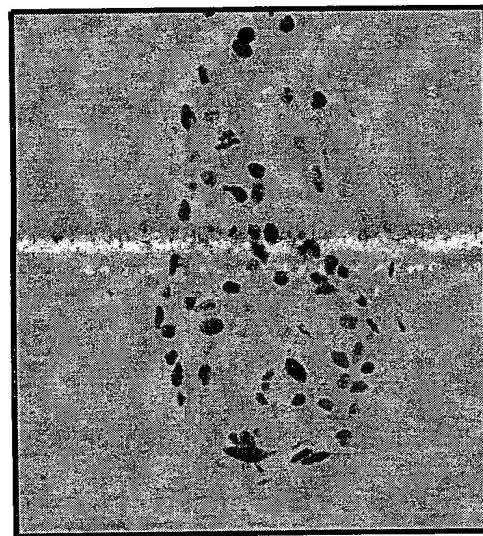


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NonTsg



K303R Tg



pH1b

18 month old virgin mice – pH1b IHC

FIG. 9